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THE USE OF *Brevibacterium linens* TO ACCELERATE THE RIPENING AND FLAVOUR DEVELOPMENT OF LOW FAT RAS CHEESE

BY

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SUMMARY

Low fat Ras cheese was manufactured from standardized pasteurized cow's milk containing 0.5, 1.0 and 1.5 fat content and from cows milk (3.8% fat) as control. The cheese curd from low fat treatments were inoculated with cell-suspension of *Brevibacterium linens* (*B. linens*) before pressing. The addition of *Brevibacterium linens* in low fat Ras cheese had no significant effect on the moisture in non fat substance (MNFS), total nitrogen and protein on dry matter basis (P/DM) contents, acidity %, total viable, lactic acid, lipolytic and proteolytic bacterial count while the water soluble nitrogen (SN), water soluble nitrogen coefficient and free amino acids contents and total volatile fatty acids were increased during ripening period. The addition of *Brevibacterium linens* in low fat Ras cheese led to the disappearance of yeast and molds. The previous results indicated that the addition of *B. linens* cell in cheese curd before pressing improved flavour in low fat Ras cheese, therefore low fat Ras cheese can be made successfully from partially removed fat milk until 1.5% fat using *B. linens*.

Key words: low fat Ras cheese / Ras cheese / cheese ripening / flavour development / *Brevibacterium linens*.

INTRODUCTION

Ras cheese, the main traditional hard cheese in Egypt, is manufactured in a high proportion under artisan production, in rural areas and small factories (El-Soda *et al.*, 2003). Ras cheese is similar to the Greek "Kefalotyri" cheese. It is made from raw cow milk or a mixture of cow and buffalo milk (El-Soda *et al.*, 2006).

Milk fat was reported to be associated with an increased risk of obesity, certain forms of cancer, atherosclerosis, coronary heart disease, elevated blood pressure and tissue injury diseases associated with lipid oxidation (Fenelon and Guinee, 2000). Technically, milk fat plays several important functions in cheese making affecting cheese firmness, adhesiveness, mouth feel and flavour development (El-Kholy, 1995 and Mann, 2000).

One of the most significant challenges facing the dairy industry in the area of

cheese manufacture is the length of the ripening period for most cheese varieties. Ras cheese is normally marketed after 4-6 months of ripening (El-Soda and Saada, 1986).

The use of microorganisms to accelerate cheese ripening can be done either directly by adding viable cells or indirectly by adding cell free extracts containing proteolytic and lipolytic enzymes to cheese milk or the curd (El-Kholy, 1995 & Adamitsch and Hample, 2000).

B. linens has long been recognized as an important dairy microorganism because of its ubiquitous presence on the surface of a variety of smear surface-ripened cheeses, such as Limburger, Munster, Brick, Tilsiter and Appenzeller. *B. linens* contributes to the formation of several flavour compounds by catabolism of amino acids producing i.e. ammonia, amines, other compounds (Hemme *et al.*,

1982), aldehydes, alcohols, 3-methylbutyric acid and caproic acid, hydrogen sulphide and methanethiol (Hemme *et al.*, 1982 and Osman, 1994). All these compounds play a fundamental role in the development of cheese flavour.

B. linens was successfully used as adjunct starter culture in the manufacture of re-

duced fat Edam and Cheddar cheese for improving their qualities (Ummadi and Weimer, 2001).

The present study aims to accelerate the ripening and flavour development of low fat Ras cheese using *B. linens* as adjunct cultures

MATERIALS AND METHODS

Materials

Fresh cow's milk was obtained from the Italian company, Salthia-El Kadema, El-Sharkea, Egypt. Freeze-dried culture (FRC-60, DVS) and (TT026), and chymosin obtained from microbial fermentation (CHY-MAX® Powder Extra NB) were obtained from CHR-Hansen's laboratories, Denmark. The FRC-60, DVS culture consisted of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* while the TT026 culture consisted of *B. linens* BL1. Fine cooking salt was obtained from the local market in Ismailia, Egypt. Plastic coat (CESKA WL 250.0185, 0.025% Natamycin - Yellow) was obtained from CSK Food Enrichment B. V., Pallasweg 1, 8930 AS Leeuwarden, The Netherlands. Analytical grade chemicals were obtained from El Nasr Pharmaceutical Chemicals Co., Egypt.

Experimental procedure

Activation of adjunct culture

B. linens was inoculated in nutrient broth medium (Biolife, Italy) plus 4% (w/v) NaCl and incubated in a shaking water bath (JULABO, SW-20C, Germany) at 27°C and 110 rpm for 48 h. The culture was kept at 4°C and subcultured every 2 weeks. The growth of *B. linens* was followed at 4 h intervals by measurement of absorbance at 620 nm using spectrophotometer (Spectronic 20 D, Milton Roy Company, USA).

Preparation of *B. linens* cell-suspension

B. linens grown in nutrient broth medium (2nd culture) by centrifugation (IEC-7000, USA) at 2326g and 4°C for 10 min. The cell pellet was washed twice with sterile saline solution (0.85%, w/v NaCl) and resuspended

in 100 mL of sterile saline solution by blending (Waring blender, Holland) at low speed for 2 min (Osman, 2003).

Ras cheese manufacture

Fresh cow's milk were standardized to casein/fat ratio (C/F) of 0.73 and then divided into 4 parts, the first was kept to serve as control (C), while the other 3 parts were standardized to 1.5, 1 and 0.5 % fat respectively. The standardized portions were divided each into two parts the first was used as control low fat Ras cheese, while the second part was inoculated with *B. linens* (CB_{1.5}, CB_{1.0} and CB_{0.5}) respectively. Milk from different batches were heated to 72°C momentary and then cooled immediately to 35°C. Ras cheeses were made from all batches according to (Hof *et al.*, 1973). Aliquot (100 mL) of *B. linens* cell suspension (8.4×10^8 CFU ml⁻¹) was added and mixed with cheese curd before molding and pressing. The curd was then molded and pressed for 24 h. The cheese was turned over every day and rubbed with dry salt for one week, then coated with plastic coat and ripened for 120 days at 14°C $\pm$ 2°C with relative humidity 80-85%. Cheese samples were examined when fresh and after 15, 30, 45, 60, 75, 90, 105 and 120 days of ripening period for chemical, microbiological and sensory properties.

Methods of Analysis

1- Chemical analysis

Moisture, fat (using Gerber method), and salt contents and titratable acidity (TA) were determined as described by (Ling, 1963), and moisture in non fat substance (MNFS) was calculated. Total nitrogen (TN) and water-soluble nitrogen (WSN) contents were determined using semi-micro kjeldahl method

(UDK126A, VELP Scientifica, Italy) as described by (Ling, 1963). Total volatile fatty acids (TVFA) was determined by the distillation method as described by (Kosikowski, 1978). Free amino acids (FAA) content was estimated using cadmium-ninhydrin method as described by (Folkertsma and Fox, 1992).

2- Microbiological analysis

Preparation of cheese samples for microbiological analysis

One gram of cheese was homogenized with 1 mL sodium citrate 20%, w/v in sterile mortar, 8 mL of physiological saline solution was added and suitable dilutions were prepared.

The total viable bacterial count (TVBC) was determined using (standard plate count agar medium, SPC) after incubation at 35°C for 3 days (Marshall, 1992). Lactic acid bacterial count (LABC) was enumerated using Elliker agar medium after incubation at 35°C for 2 days (Elliker *et al.*, 1956). Antibiotic standard plate count agar medium (Marshall, 1992) was used for the enumeration of yeast and molds after incubation at 25°C for 4 days under aerobic condition.

The count of the lipolytic and proteolytic bacteria were determined by plating suitable dilutions (in duplicates) on nutrient agar medium containing 1% sterilized oil and

skim milk (5%, w/v) respectively. The plates were incubated at 37°C / 4 days. For lipolytic bacteria the plates were covered with copper sulphate solution (20%, w/v) for 10 min, after which the copper sulphate solution was discarded, and the plates were examined to detect lipolytic green color colonies (Osman, 1987). For proteolytic bacteria the plates were covered with HCl (10%, v/v) for 1 min then discarded. The clear zones around the colonies refer to proteolytic bacteria (Osman, 1987).

Sensory evaluation

The cheeses were scored during ripening for appearance, body & texture and flavour by a regular score panels of the staff members of the dairy department. Scoring was carried out for flavour out of 50 points, body & texture out of 40 points and appearance out of 10 points (Pappas *et al.*, 1996).

Statistical analysis

Each experiment was independently replicated three times in a factorial design (2 factors $\times$ 3 replications). All analyses and enumerations were done in duplicate. All data were analyzed by ANOVA using the general models procedure of (COSTAT, 1998). Differences among means were tested for significance ($P < 0.01$) by Duncan's multiple range tests.

RESULTS AND DISCUSSION

Chemical composition

The MNFS is essentially a ratio of water to protein and is related to firmness (Van Hekken and Tunick, 2002), and considered a better predictor of texture than moisture or fat contents. The values of MNFS content of C, CB_{1.5} and C_{1.5} treatments were significantly ($p < 0.01$) higher than in C_{1.0}, CB_{1.0}, C_{0.5} and CB_{0.5} treatments Figure (1). The statistical analysis of MNFS indicated that there were a significant differences ($p < 0.01$) between control cheese (C) and cheese treatments (C_{1.5} & CB_{1.5}, C_{1.0} & CB_{1.0} and C_{0.5} & CB_{0.5}). Data reveal that, moisture in non fat substance (MNFS) of cheese decreased proportionally as the fat content of starting cheesemilk decreased /or as the ripening period prolonged

($p < 0.01$). These results could be attributed to the high moisture content of low fat cheese which were significantly ($p < 0.01$) higher than that of the full fat cheese. Similar finding were reported by Banks (2004) & Küçüköner and Haque (2006). The use of *B. linens* did not lead to any significant differences ($p < 0.01$) in the MNFS content of low fat Ras cheese.

Figure (2), shows that the addition of *B. linens* had no significant ($p < 0.01$) effect on acidity %. The data indicated that the acidity % reached a maximum after 2 mon of ripening and then decreased slightly thereafter. The decrease in acidity % could be attributed to the formation of basic nitrogen compounds and

reduction of lactic acid formation (Osman, 2003). Similar trend was obtained by Awad (2006). Also Osman (2003) found that the acidity % reached a maximum after 1 month of ripening and then decreased slightly thereafter.

The changes of total nitrogen (TN), protein on dry matter basis (P/DM), water soluble nitrogen (WSN) and water soluble nitrogen coefficient (WSN/TN) of low fat Ras cheese as a function of the use of *B. linens* as an adjunct starter culture are shown in Figure (3).

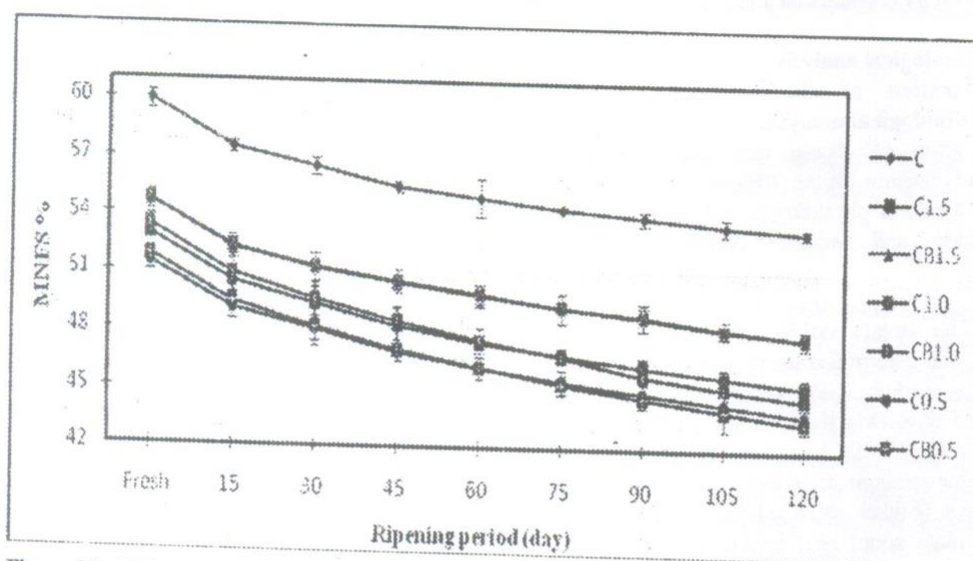


Figure (1) : Effect of using *B. linens* on moisture in non-fat substances (MNFS)

C: control full fat Ras cheese; C_{1.5}: control low fat Ras cheese (1.5% fat); CB_{1.5}: low fat Ras cheese (1.5% fat) with *B. linens*; C_{1.0}: control low fat Ras cheese (1% fat); CB_{1.0}: low fat Ras cheese (1% fat) with *B. linens*; C_{0.5}: control low fat Ras cheese (0.5% fat); CB_{0.5}: low fat Ras cheese (0.5% fat) with *B. linens*. Bars (⊕) represented standard deviations.

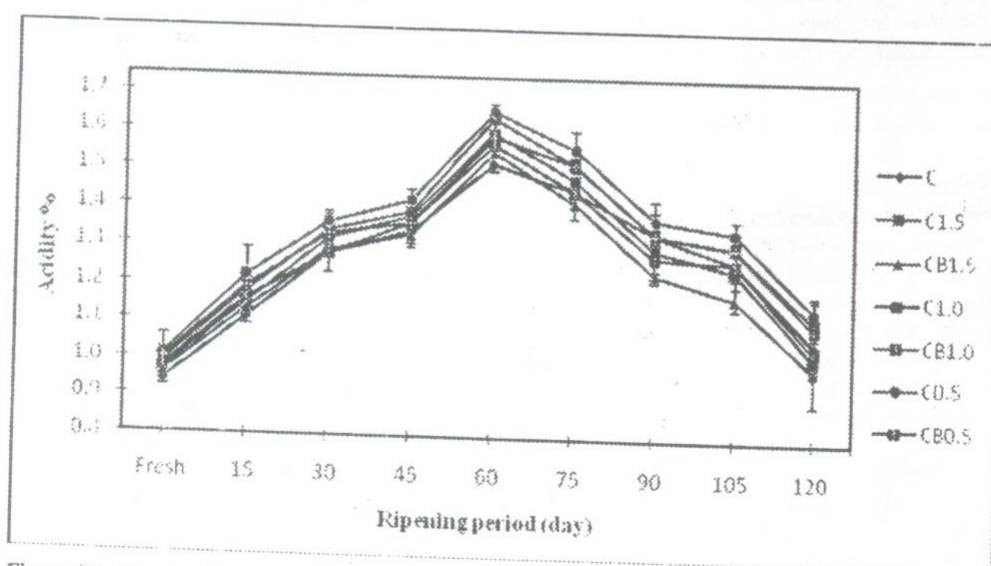


Figure (2) : Changes in acidity % during ripening period of Ras cheese made from different fat contents using *B. linens*
See legend Fig 1

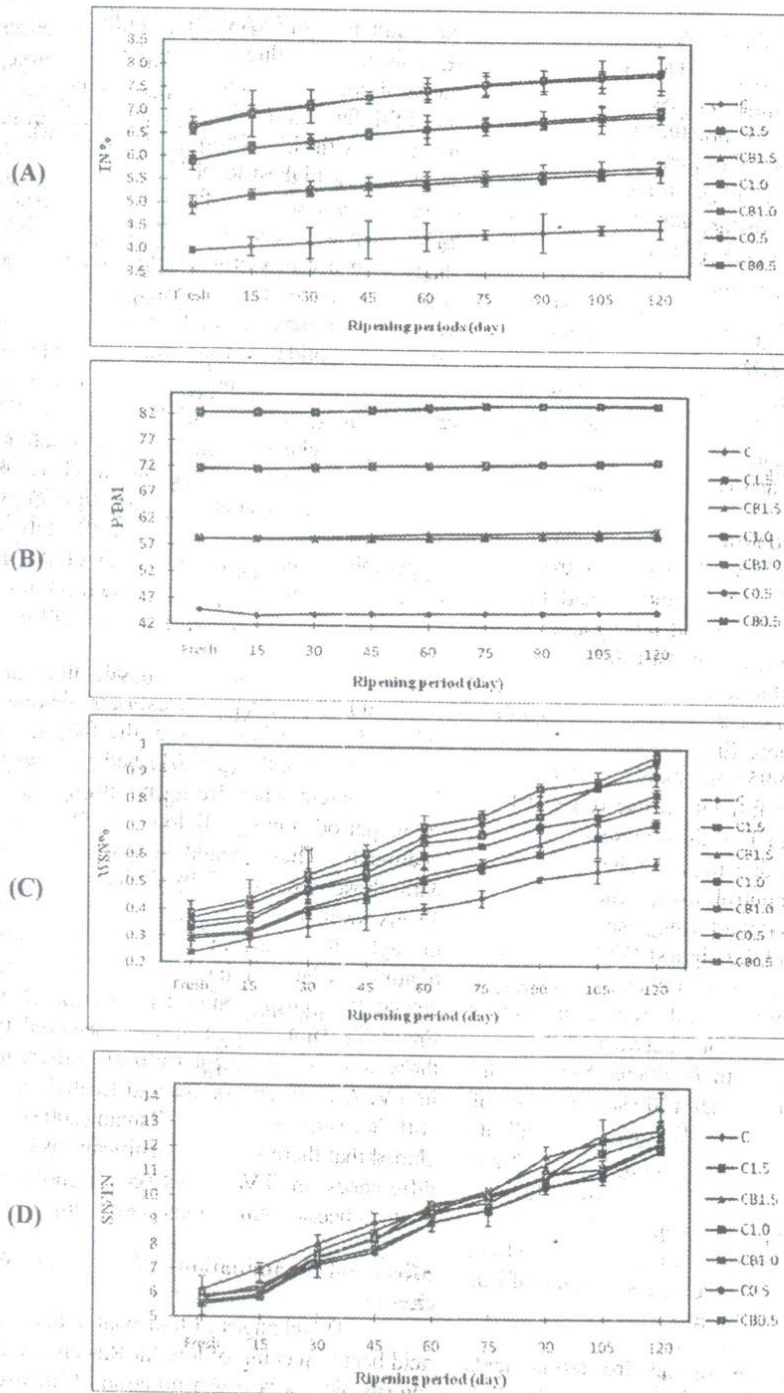


Figure 3 : Effect of using *B. linens* on (A) total nitrogen content, (B) protein on dry matter basis (P/D.M), (C) water soluble nitrogen content (WSN) and (D) water soluble nitrogen coefficient (WSN/TN) during ripening period of Ras cheese made from different fat content.

See legend Fig 1

All low fat Ras cheese treatments can be characterized by high TN content than that from full fat milk cheese (control). The changes in TN and moisture contents of low fat Ras cheeses during ripening were related to each other. The decrease in moisture contents during ripening was accompanied with an increase in TN contents (Osman, 2003). It is obvious from these results that the addition of *B. linens* in low fat Ras cheese had no significant ($p < 0.01$) effect on TN content. Protein on dry matter basis (P/DM) contents of low fat Ras cheese were more than full fat Ras cheese. The addition of *B. linens* in low fat Ras cheese had no significant ($p < 0.01$) effect on protein on dry matter basis (P/DM) content.

The control with full fat cheese (C) had the lowest WSN %, while control low fat Ras cheese (C_{1.5}, C_{1.0}, and C_{0.5}) had higher WSN % when fresh and throughout ripening period compared to control full fat cheese (C). This may be due to the higher TN content of control low fat cheese compared to control full fat cheese. These findings were confirmed by El- Kholy (1995) and Salem (1998). As for WSN/TN, the full fat cheese (C) had higher WSN/TN than low fat cheese (C_{1.5}, C₁, and C_{0.5}). This is due to its higher ripening rate compared to control low fat cheese. El- Kholy (1995) reported that the cheese with the higher fat content had the highest WSN as a percentage of total nitrogen. However, there were no significant ($p < 0.01$) differences in WSN % and WSN/TN ratio between control and cheese treated with *B. linens*. Similar results were found by Osman (2003). The addition of *B. linens* in low fat Ras cheese in all treatments increased WSN and WSN/TN ratio significantly ($p < 0.01$) comparing to full fat cheese during ripening. Effat *et al.* (1992) found that the addition of proteinase extract from *B. lienes* increased WSN/TN ratio of Ras cheese during ripening.

The changes in total free amino acids (absorbance at 507 nm/100 μ L) are shown in Figure (4). The data indicated that the FAA in all cheeses significantly ($p < 0.01$) increased as ripening period progressed and this increase was more pronounced at the 4th month of ripening period. As it is expected, the low fat Ras cheese (C_{1.5}, C_{1.0}, and C_{0.5}) had higher

concentrations of FAA than full fat cheese (C). This may be due to the higher protein content of control low fat cheese compared to control full fat cheese. These results are in full agreement with El- Kholy (1995) who reported that the highest level of free amino acids was found in low fat cheese as a result of higher protein content. These results could be changed if it proportional to the total nitrogen content of the cheese. The liberation of FAA in ripening may be due to the release of intracellular peptidases from dead and lysed starter cells (Folkertsma and Fox 1992). There were significant ($p < 0.01$) differences in the FAA content of cheese from different treatments when used *B. linens* in low fat Ras cheeses. The increase of FAA content during ripening period were expected, due to the effect of the proteolytic enzymes from rennets, starter cultures, milk enzymes and other factors on proteolysis of cheese protein (Fox *et al.*, 1993).

Changes in total volatile fatty acids (mL NaOH 0.1 M/10g cheese) are shown in Figure (5). As it is expected, the full fat Ras cheese significantly ($p < 0.01$) had the highest TVFA content when fresh and along the ripening period among all low fat Ras cheese treatments. These results are in agreement with those reported by Salem (1998). The TVFA were increased significantly ($p < 0.01$) throughout the ripening period in all cheese treatments. The obtained results are in accordance with those found by Osman (2003). From the same Figure, it was observed that there were a significant ($p < 0.01$) differences in TVFA between control and treated cheese with *B. linens*. In contrast, Osman (2003) concluded that there were no significant ($p < 0.05$) differences in TVFA between control and treated cheese with *B. linens* cell culture.

Microbial examination of low fat Ras cheese

The changes of total viable and lactic acid bacterial count of low fat Ras cheese during ripening period as a function of the use of *B. linens* are shown in Figure (6). There were no significant ($p < 0.01$) differences in the numbers of total viable (TVBC) and lactic acid bacterial count (LABC) between control and *B. linens* treated cheese. These results are in agreement with Osman (2003). The data

indicated that the reduction in the fat content of Ras cheese milk was associated with proportional reduction in the counts enumerated in the resultant cheese of TVBC. *B. linens* treated cheeses had higher TVBC than untreated cheeses. TVBC and LABC in all cheese treatments were gradually decreased until the end of ripening period. There were

significant ($p < 0.01$) decrease in TVBC and LABC in all cheeses during ripening. These results are in agreement with (Osman, 2003 and Younis *et al.*, 2004). The reduction of TVBC and LABC may be due to the increase of salt, acidity % and decrease of pH values during ripening period.

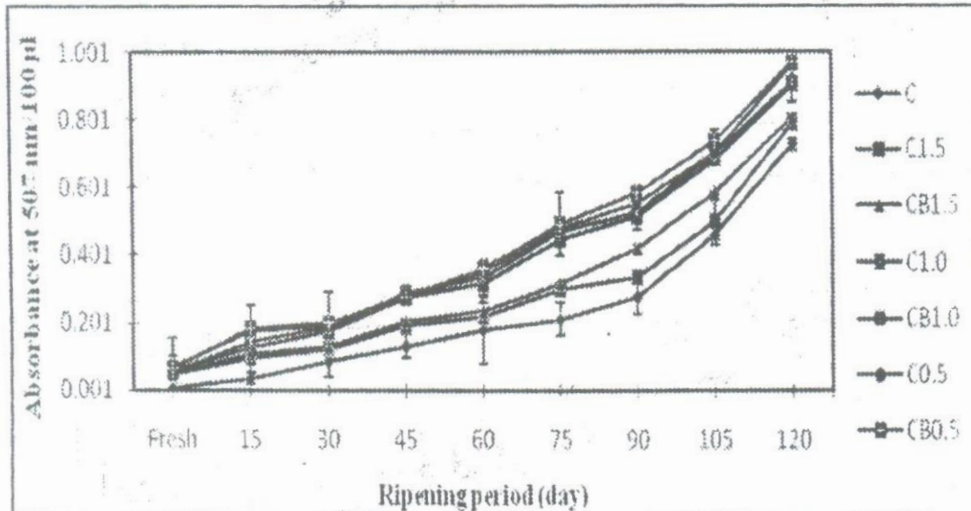


Figure (4): Effect of using *B. linens* on free amino acids (expressed as $A_{507}/100 \mu\text{l}$) content during ripening period of Ras cheese made from different fat contents. See legend Fig 1

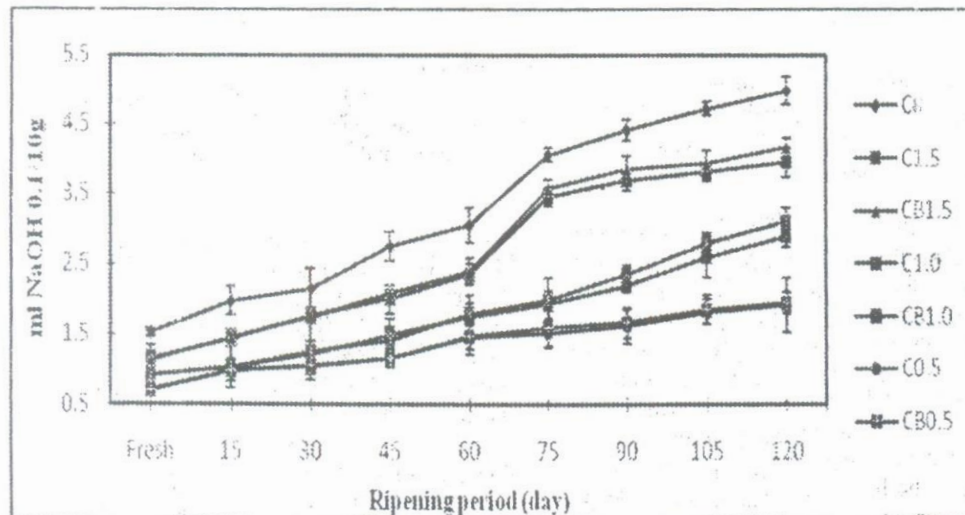


Figure (5): Effect of using *B. linens* on total volatile fatty acids (expressed as ml NaOH 0.1 M/10 g cheese) content during ripening period of Ras cheese made from different fat contents. See legend Fig 1

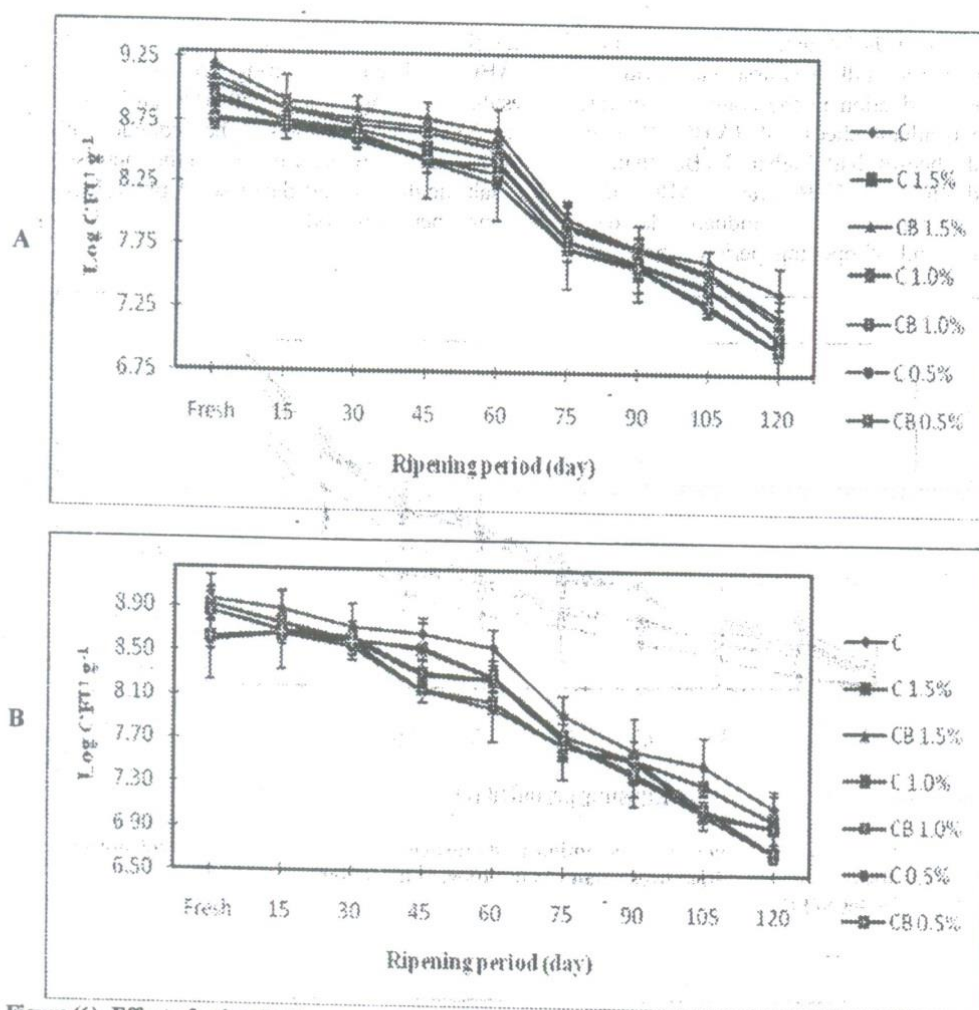


Figure (6): Effect of using *B. linens* on total viable bacterial count (A) and lactic acid bacterial count (B) during ripening period of Ras cheese made from different fat contents. See legend Fig 1

There were no significant ($p < 0.01$) differences in numbers of lipolytic or proteolytic bacterial count between control and *B. linens* treated cheese Figure (7). They were increased during ripening period, until the day 15th then declined gradually until the end of experimental period. These results are in agreement with Younis *et al.* (2004). The proteolytic bacterial count (Figure, 7) were generally present in higher numbers in all fresh cheeses than the lipolytic bacteria. These results are in agreement with Salem (1998). The reduction of the lipolytic and proteolytic bacterial counts may be due to the increase of salt, acidity % and decrease of pH values during ripening period.

It was obvious from the data (data not showed) that the addition of *B. linens* in low fat Ras cheese led to the disappearance of yeast and molds. These results are in agreement with Osman (1999) who reported that *B. linens* isolated from Domiati cheese inhibited the growth of different fungi.

Organoleptic properties

The use of *B. linens* significantly ($p < 0.01$) increased the total scores gained by the low fat cheese during ripening Table (1). The control (C) gained the highest total scores followed by CB_{1.5}, C_{1.5}, CB_{1.0}, C_{1.0}, CB_{0.5} and C_{0.5}. The addition of *B. linens* improved the flavour, body and texture compared to control

low fat Ras cheese. These results are in agreement with Rattary and Fox (1999) who mentioned that the use of *B. linens* cultures as adjunct cultures had a very positive effect on cheese flavour. It is clear that CB_{1.5} gained the highest scores for sensory evaluation which possessed a distinct flavour and very good

body & texture and scored a total score 90.67 points at the end of ripening period compared to C_{1.5} which possessed a slight flavour and hard and slight grainy body & texture and scored a total score 88.33 points at the end of ripening period. These results are in agreement with Osman (2003).

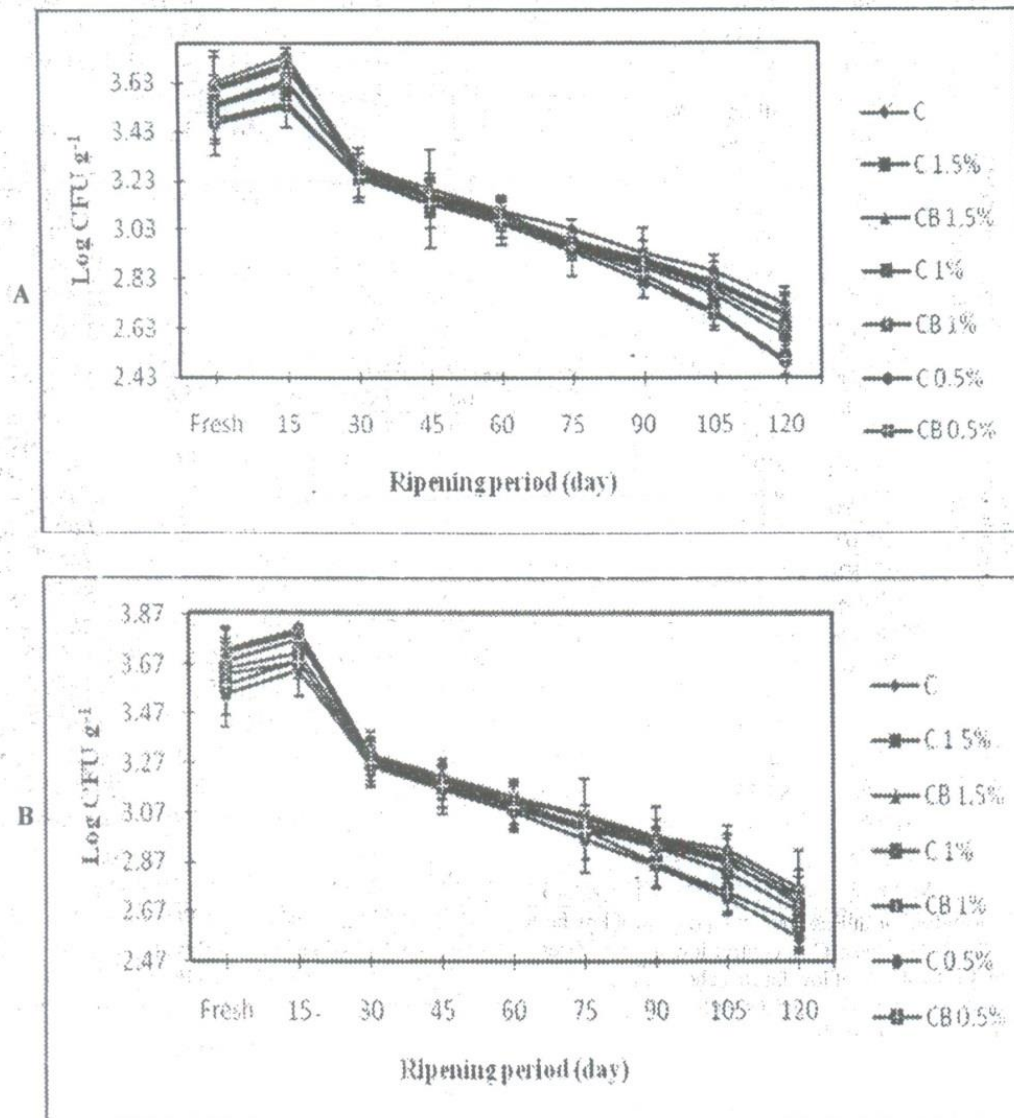


Figure (7): Effect of using *B. linens* on Lipolytic (A) and Proteolytic (B) bacterial count during ripening period of Ras cheese made from different fat contents. See legend Fig 1

Table (1): Organoleptic properties of low fat Ras cheese manufactured with *B. linens*

Prop- erties	Ripening period (days)	Treatments							Mean
		C	C _{1.5}	CB _{1.5}	C _{1.0}	CB _{1.0}	C _{0.5}	CB _{0.5}	
Flavour (50)	30	33.00	32.00	32.00	31.00	31.00	28.33	28.33	30.81 ^g
	45	36.00	34.33	34.33	33.33	33.33	29.67	29.67	32.96 ^f
	60	39.33	37.33	38.00	36.00	37.00	31.33	31.33	35.76 ^e
	75	41.00	38.00	38.33	37.33	37.67	33.00	33.00	36.90 ^d
	90	43.00	41.00	42.00	40.00	40.67	34.00	34.33	39.29 ^c
	105	44.33	41.33	42.33	40.67	41.33	35.33	35.67	40.14 ^b
	120	45.67	42.67	44.33	41.67	42.00	36.00	36.67	41.29 ^a
	Mean [*]	40.33 ^a	38.10 ^{bc}	38.77 ^b	37.14 ^d	37.58 ^{cd}	32.52 ^e	32.71 ^e	
Body and texture (40)	30	32.00	31.33	31.33	30.67	30.67	26.00	24.00	29.43 ^f
	45	33.33	32.67	32.67	31.33	31.33	27.00	26.00	30.62 ^e
	60	34.67	33.67	33.67	32.33	32.67	27.00	25.00	31.29 ^{de}
	75	35.00	34.33	34.67	33.00	33.67	27.67	26.00	32.05 ^{cd}
	90	36.00	35.33	35.67	33.00	33.67	28.00	26.33	32.57 ^{bc}
	105	37.00	36.67	37.00	34.00	34.67	26.33	26.00	33.10 ^{ab}
	120	37.67	37.00	37.33	35.00	35.33	26.67	27.33	33.77 ^a
	Mean [*]	35.10 ^a	34.43 ^a	34.62 ^a	32.76 ^a	33.14 ^a	26.96 ^a	25.81 ^a	
Appearance (10)	30	7.33	7.33	7.33	7.00	7.00	4.33	4.33	6.38 ^e
	45	7.33	7.33	7.33	7.00	7.00	4.67	4.67	6.48 ^e
	60	8.00	7.67	7.67	7.00	7.33	4.67	4.67	6.71 ^{de}
	75	8.00	8.00	8.00	7.33	7.67	5.00	5.00	7.00 ^{cd}
	90	8.33	8.00	8.33	7.67	8.00	5.00	5.33	7.24 ^{bc}
	105	9.00	8.33	8.67	8.00	8.33	5.00	5.67	7.57 ^{ab}
	120	9.00	8.67	9.00	8.33	8.67	5.00	5.67	7.76 ^a
	Mean [*]	8.14 ^a	7.90 ^{ab}	8.05 ^{ab}	7.48 ^c	7.71 ^{bc}	4.81 ^d	5.05 ^d	
Total (100)	30	72.33	70.67	70.67	68.67	68.67	58.67	56.67	66.62 ^g
	45	76.67	74.33	74.33	71.67	71.67	61.33	60.33	70.05 ^f
	60	82.00	78.67	79.33	75.33	77.00	63.00	61.00	73.76 ^e
	75	84.00	80.33	81.00	77.67	79.00	65.67	64.00	75.95 ^d
	90	87.33	84.33	86.00	80.67	82.33	67.00	66.00	79.10 ^c
	105	90.33	86.33	88.00	82.67	84.33	66.67	67.33	80.81 ^b
	120	92.33	88.33	90.67	85.00	86.00	67.67	69.67	82.81 ^a
	Mean [*]	83.57 ^a	80.43 ^b	81.43 ^b	77.38 ^c	78.43 ^c	64.29 ^d	63.57 ^d	

C: control full fat Ras cheese; C_{1.5}: control low fat Ras cheese (1.5% fat); CB_{1.5}: low fat Ras cheese (1.5% fat) with *B. linens*; C_{1.0}: control low fat Ras cheese (1% fat); CB_{1.0}: low fat Ras cheese (1% fat) with *B. linens*; C_{0.5}: control low fat Ras cheese (0.5% fat); CB_{0.5}: low fat Ras cheese (0.5% fat) with *B. linens*.

*a, b, c, d, e, f and g: means with the same letter among the treatments or ripening period are not significantly different (p < 0.01)

CONCLUSION

Finally there were a clear relation between the data from the chemical analysis of all cheeses and the assessment of their organoleptic quality. We can concluded that the addition of *B. linens* cell in cheese curd before

pressing accelerated the development of flavour in low fat Ras cheese. Low fat Ras cheese could successfully made from partially removed fat milk until 1.5% fat using *B. linens*.

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استخدام *Brevibacterium linens* لاسراع تسوية و تحسين نكهة الجبن الراس المنخفض في نسبة الدهن

صنع جبن راس منخفض في نسبة الدهن من لبن بقرى مبستر عدلت به نسبة الدهن الى ١.٥ ، ١.٥ و ٣.٨% (معاملة مقارنة). لقيت الخثرة في كل معاملات الجبن بمعلق *B. linens* قبل الكبس. اوضحت النتائج أن إضافة *B. linens* لا يسبب وجود فروق معنوية في محتوى الرطوبة، النيتروجين الكلي، البروتين الكلي في المادة الجافة، الحموضة والعدد الكلي للبكتريا والعدد الكلي لبكتريا حمض اللاكتيك واعداد البكتريا المحللة للدهن والبروتين بينما حدثت زيادة في معامل النيتروجين الدائب ومحتوى النيتروجين الدائب في الماء والاحماض الامينية الحرة والاحماض الدهنية الكلية الطيارة خلال فترة التسوية. إضافة *B. linens* في الجبن المنخفض في نسبة الدهن أدى إلى اختفاء الخمائر والفطريات. ومن النتائج السابقة يتضح أن إضافة *B. linens* إلى الخثرة قبل الكبس أدى إلى تحسين نكهة الجبن الراس منخفض الدهن، وعلى ذلك يمكن صناعة جبن راس منخفض الدهن بنجاح من لبن منخفض في نسبة الدهن (١.٥%) وباستخدام *B. linens*.